EXHIBIT B

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A directory of human germ-line V_κ segments reveals a strong bias in their usage

From the genomic DNA of a single individual, we have amplified, cloned and sequenced 37 human germ-line V_κ segments. Four of these segments were new. We then compiled a comprehensive directory of all germ-line V_κ segments and identified 50 different sequences with open reading frames. Comparison with 236 rearranged sequences revealed that no more than 24 of these germ-line sequences could be assigned rearranged counterparts, that some of these were rarely used, and that only about 11 sequences are used frequently. This suggests that the expressed V_κ repertoire is mainly derived from a limited number of segments. Most surprisingly, the J_κ-distal region of the locus appears to be rarely used: we could unambiguously assign 162 rearranged sequences to V_κ segments of the J_κ-proximal region, but only 5 to segments of the J_κ-distal region.

1 Introduction

Recently antibodies of predefined binding specificity have been derived from repertoires of associated heavy and light chain variable (V) domains displayed on the surface of filamentous phage ([1]; for review see [2]). The process mimics production of antibodies by the immune system and bypasses hybridoma technology and immunization [3, 4]. Diverse repertoires of V domains have been provided by PCR amplification [5] of heavy and light chain V genes [6] from populations of lymphocytes. Alternatively the repertoires have been provided by *in vitro* rearrangement of cloned V segments [7]. To provide a bank of cloned V gene segments, we have amplified and sequenced V_H and V_λ segments from genomic DNA, leading to the isolation of almost all the functional human V_H segments [8] and many of the human V_λ segments [9]. Here we have attempted to gather a large selection of human germ-line V_κ segments using the same strategy. After submission of this report, a number of new V_κ segments were sequenced and mapped [10, 11]. For completeness we have included this information in our revised paper.

The human immunoglobulin light chain kappa (κ) locus is located on the short arm of chromosome 2 (2p11-12) [12, 13] and consists of a C_κ gene, 5 J_κ segments and at least 76 mapped germ-line V_κ segments [11, 14]. The V_κ segments may be divided by sequence homology [15] into three main subgroups, I–III, and several smaller subgroups (IV, V, VI and VII) [11, 16–18]. The portion of the locus harboring the majority of V_κ segments is thought to have arisen from a duplication event [19, 20]: 36 segments are located in the J_κ-distal region and 40 in the J_κ-proximal region [11, 14, 21, 22]. Segments are clustered in four distinct regions, A, B, L and O (Fig. 1) [18]. The A, L and O clusters are found within both the J_κ-distal and the J_κ-proximal regions, the B cluster only within the J_κ-proximal region.

Of the 76 mapped V_κ segments within the major locus, 57 have published sequences. Of these 57 segments, 48 have alleles with open reading frames (these correspond to 50 different sequences due to there being some segments from the J_κ-proximal and J_κ-distal regions which are identical and other segments which have multiple alleles) and 9 have frame shifts, stop codons or incomplete exons and are, therefore, regarded as pseudogenes. Most of the remaining 19 segments are also known to be pseudogenes [10, 16, 17, 19, 20, 23–36] (Fig. 1). There are also a number of orphon V_κ segments which have been mapped outside the major κ locus: W1-W11 [37, 38], V108 [39], Chr22-1 to Chr22-5 [40, 41], Chr1 [41], cos 118 [41] and Z1-Z4 [42, 43]. There are two further segments with open reading frames that have not been mapped (LFVK5 and LFVK431; L. Foroni, personal communication).

To clone the functional V_κ segments, we assembled a database of published germ-line sequences and then designed PCR primers to amplify each of the different subgroups. A germ-line V_κ segment consists (5′ to 3′) of a leader sequence (L), a leader intron, a continuation of the leader sequence (L′) and the V_κ exon (Fig. 2). Within the exon there are three framework regions (FR) and three complementarity-determining regions (CDR). Adjoining the 3′ end of the exon is a highly conserved heptamer recombination signal and a less conserved nonamer region, the two separated by 11–14 nucleotides. Foward PCR primers were based in the heptamer region and back primers on the overlap between the leader intron and L′ region (Fig. 2 and Table 1). Using these primers we amplified, cloned and sequenced germ-line V_κ segments from the genomic DNA extracted from the peripheral white blood cells of a single donor (DP).

2 Materials and methods

2.1 Primer design

Forward and back primers were designed for the V_κI, II, III, IV and VI subgroups from alignments of published germ-line sequences. For the V_κI subgroup, three cluster-specific

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Abbreviations: CDR: Complementarity-determining region **FR:** Framework region

Key words: Human immunoglobulin kappa (V_κ) segments

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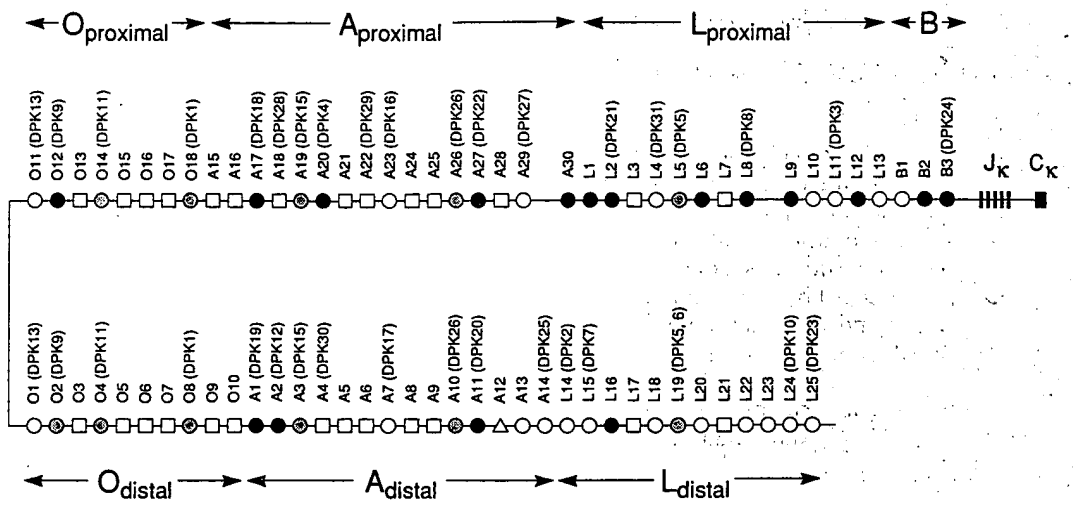


Figure 1. Schematic representation of the human immunoglobulin κ locus (after [11, 21]). Germ-line $V_κ$ segments with open reading frames are represented by circles. The segments A4, A5, A18, A22, A28, L3, L7, L17 and L21 have frame shifts, stop codons or incomplete $V_κ$ exons and are, therefore, regarded as pseudogenes (squares). The remaining 19 segments have either not been sequenced (triangle) or are known to be pseudogenes (squares). The segments L4 and L16 have a number of alleles, some with open reading frames and others with stop codons. Where a segment has been seen rearranged *in vivo*, the circle has been filled (black or shaded). If the rearranged sequences could not be unambigously assigned to segments of the $J_κ$-proximal or $J_κ$-distal regions, the corresponding segments from both regions have been shaded. Sequences from this study have been attributed their DPK codes (see text).

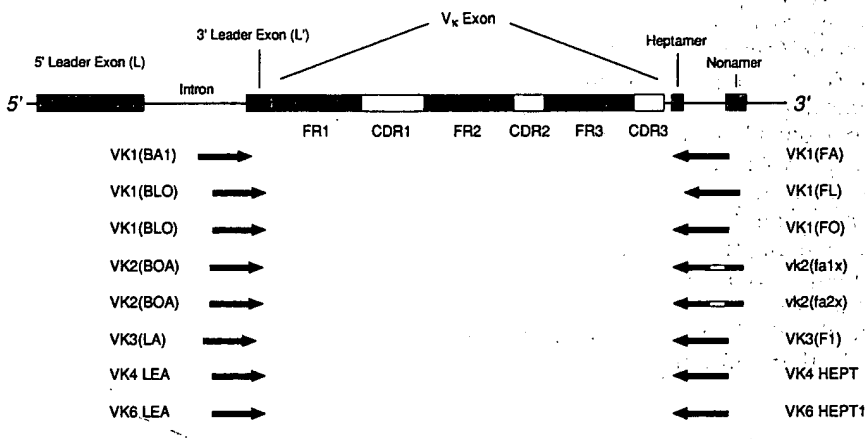


Figure 2. Location of PCR primers for amplification of the human germ-line $V_κ$ segment.

Table 1. Primers used for PCR amplification of the $V_κ$ exon

	Primer	Sequence (5' to 3')
$V_κI$	VK1(FA)	GCT CTA GAC GGG CTT GTA TCA CAG TG
	VK1(FL)	GCT CTA GAG TT(CT) (AG)GG T(GT)(GT) GTA ACA CT
	VK1(FO)	GCT CTA GAA TG(AC) CTT GT(TA) ACA CTG TG
	VK1(BA1)	CCC CCA AGC TTT GTT CCT AAT ATC AGA TA
	VK1(BLO)	CCC CCA AGC TTA ATC (TG)CA GGT (GT)CC AGA TG
$V_κII$	vk2(fa1x)	GAG GTT TTC TAG A(TG)G (GA)(GT)(CT) TGT A(GC)C ACT GTG
	vk2(fa2x)	GAG GTT TTC TAG AAG (GA)(GT)(CT) TGT A(GC)C ACT GTG
	VK2(BOA)	CCC CCA AGC TT(TA) A(TC)T TCA GGA TCC AGT G
$V_κIII$	VK3(F1)	GGA ATT CT(CT) A(TA)G (CT)TG AAT CAC TGT G
	VK3(LA)	CCC CCA AGC TTT CCA AT(TC) T(CT)(AG) GAT ACC AC
$V_κIV$	VK4 HEPT	GCT CTA GAC GAG GCT GAA GCA CTG TG
	VK4 LEA	CCC CCA AGC TTA CTA CAG GTG CCT ACG GG
$V_κVI$	VK6 HEPT1	GCT CTA GAG GGT TGT A(GA)C ACA GTG TG
	VK6 LEA	CCC CCA AGC TTT TTT CAG CCT CCA GGG GT

forward primers were designed, VK1(FA), VK1(FL), and VK1(FO), corresponding to the A, L and O clusters, respectively. Two cluster-specific back primers, VK1(BA1) and VK(BLO), corresponding to the A cluster and the combined B and L clusters were also used. *Hind*III restriction sites were incorporated into the 5′ end of all back primers. All forward primers had *Xba*I restriction sites at the 5′ end, except VK3(F1) which had an *Eco*RI site and the V_κII primers, where the *Xba*I site was introduced into the middle of the primer to replace a particularly degenerate section. The primers were used in pairs as indicated in Fig. 2.

2.2 PCR amplification and sequencing

PCR amplification was performed according to [8] with the following modifications. Products from the PCR amplifications and restriction enzyme digests were purified using Magic™ PCR Preps (Promega, Madison, WI). Inserts from plaques picked from a TYE [44] plate were amplified with an M13mp19-specific primer pair using the PCR (25 cycles, each cycle consisting of 1 min at 94 °C, 1 min at 55 °C, 30 s at 72 °C; at the end of 25 cycles there was a final extension at 65 °C for 5 min). Sequencing was performed using an M13-specific primer, Taq polymerase and fluorescent dideoxy chain terminators [45]. The sequences were analyzed on an Applied Biosystems 373A Automated DNA Sequencer (Foster City, CA).

3 Results

Amplification using the subgroup-specific primers with suitable adjustment of the annealing temperature gave single PCR bands of varying intensities. Primer pairs were found to be specific for their respective subgroups. We sequenced 142 clones (62 V_κI, 60 V_κII, 14 V_κIII, 1 V_κIV and 5 V_κVI). From these, 37 V_κ sequences were identified. Excluding those identical to mapped orphon segments (see below) 27 segments had open reading frames (DPK1-DPK27): 24 were identical to published sequences and 3 segments, DPK2, DPK14 and DPK23, were new. DPK2 was most similar to L1/HK137 (14 nucleotide changes), DPK14 to the pseudogene A5 (insertion of one nucleotide and 3 changes), and DPK23 to L10/Vh (8 nucleotide changes). The remaining segments are identical to the pseudogenes

Table 2. Assignment of rearranged V_κ sequences to their closest germline counterparts

	Germline sequence	No. of rearranged sequences[a]	Closest rearranged sequence Δ(N, P)[b]
V_κI	O8/O18/DPK1	21	(0, 0)
	A30	3	(5, 2)
	L1/HK137	1	(15, 8)
	A20/DPK4	9	(0, 0)
	L5/L19[1]/Vb/Vb′/V4b/DPK5	4	(3, 0)
	L8/Vd/DPK8	10	(1, 0)
	L9/Ve	3	(0, 0)
	L12[1]/HK102/V1	1	(37, 17)
	L12[2]	12	(5, 1)
	O12[1]/V3b	1	(2, 1)
	O2/O12[2]/DPK9	30	(0, 0)
	O4/O14/LFVK19H/DPK11	1	(11, 6)
		96	
V_κII	A2/DPK12	1	(9, 8)
	A3/A19/DPK15	11	(0, 0)
	A17/DPK18	11	(1, 0)
	A1/DPK19	1	(0, 0)
		24	
V_κIII	A11/humkv305/DPK20	1	(3, 2)
	L16/humkv328/humkv328h2	2	(3, 3)
	L2/humkv328h5/DPK21	20	(1, 0)
	A27/humkv325/Vκ RF/DPK22	48	(0, 0)
	L6/Vg	19	(0, 0)
		90	
V_κIV	B3/VkIV/DPK24	22	(1, 1)
V_κV	B2/EV15	2	(0, 0)
V_κVI	A10/A26/DPK26	2	(3, 1)

a) Number of rearranged sequences assigned to their closest germline counterpart.

b) Δ(N, P) denotes the number of nucleotide (N) and amino acid residue changes (P).

				L1				L2			L3
				FR1	CDR1	FR2	CDR2	FR3			CDR3
				1 10 20	30	40	50	60	70	80	90
$V_\kappa I$	7	R0	**O8/O18**[33]/DPK1	DIQMTQSPSSLSASVGDRVTITC	QASQDISN YLN	WYQQKPGKAPKLLIY	DASNLET	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC			QQYDNLP
	7		**L14**[10]/DPK2	NIQMTQSPSAMSASVGDRVTITC	RARQGISN YLA	WFQQKPGKVPKHLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC			LQHNSYP
	7		**L15**^{1}[10]/HK101[31]/HK146/HK189[19]	DIQMTQSPSSLSASVGDRVTITC	RARQGISS WLA	WYQQKPEKAPKSLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQYNSYP
	7		**L11/Vf**[33]/DPK3	AIQMTQSPSSLSASVGDRVTITC	RASQGIRN DLG	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			LQDYNYP
	7	R2	**A30**[10]	DIQMTQSPSSLSASVGDRVTITC	RASQGIRN DLG	WYQQKPGKAPKRLIY	AASSLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC			LQHNSYP
	7		LFVK5[*]	DIQMTQSPSSLSASVGDRVTITC	RASQGISN NLN	WYQQKPGKTPKLLIY	AAPSLQS	GIPSRFSDSGSGADYTLTIRSLQPEDFATYYC			QQSDSTP
	7		LFVK431[*]	DIQMTQSPSSLSASVGDRVTITC	RASQGISN YLA	WFQQKPGKAPKSLIY	AASSLQS	GVPSKFSGSGSGTDFTLTISSLQPEDFATYYC			QQYNSYP
	7	R8	**L1**[10]/HK137[19]	DIQMTQSPSSLSASVGDRVTITC	RASQGISN YLA	WFQQKPGKAPKSLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQYNSYP
	7	R0	**A20**[17]/DPK4	DIQMTQSPSSLSASVGDRVTITC	RASQGISN YLA	WYQQKPGKVPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC			QKYNSAP
	7		**L18**^{1}[10]/Va''[20]	AIQLTQSPSSLSASVGDRVTITC	RASQGISS ALA	WYQQKPGKAPKLLIY	DASSLES	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQFNNYP
	7		**L4/L18**^{2}/Va'/V4a[35]	AIQLTQSPSSLSASVGDRVTITC	RASQGISS ALA	WYQQKPGKAPKLLIY	DASSLES	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQFNSYP
	7	R0	**L5/L19**^{1}[10]/Vb/Vb'/V4b[35]/DPK5	DIQMTQSPSSVSASVGDRVTITC	RASQGISS WLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQANSFP
	7		**L19**^{2}/Vb''[35]/DPK6	DIQMTQSPSSVSASVGDRVTITC	RASQGISS WLA	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQANSFP
	7		**L15**^{2}[10]/HK134/HK166[19]/DPK7	DIQMTQSPSSLSASVGDRVTITC	RASQGISS WLA	WYQQKPEKAPKSLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQYNSYP
	7	R0	**L8**/Vd[35]/DPK8	DIQLTQSPSFLSASVGDRVTITC	RASQGISS YLA	WYQQKPGKAPKLLIY	AASTLQS	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC			QQLNSYP
	7	R0	**L9**/Ve[35]	AIRMTQSPSSFSASTGDRVTITC	RASQGISS YLA	WYQQKPGKAPKLLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC			QQYYSYP
	7	R17	**L12**^{1}/HK102[31]/V1[25]	DIQMTQSPSTLSASVGDRVTITC	RASQSISS WLA	WYQQKPGKAPKLLIY	DASSLES	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC			QQYNSYS
	7	R1	**L12**^{2}[10]	DIQMTQSPSTLSASVGDRVTITC	RASQSISS WLA	WYQQKPGKAPKLLIY	KASSLES	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC			QQYNSYS
	7	R1	**O12**^{1}/V3b[29]	DIQMTQSPSFLSASVGDRVTITC	RASQSISS YLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQGYSTP
	7	R0	**O2/O12**^{2}[29]/DPK9	DIQMTQSPSSLSASVGDRVTITC	RASQSISS YLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC			QQSYSTP
	7		**L24**[10]/Ve''/V13[25]/DPK10	VIWMTQSPSLLSASTGDRVTISC	RMSQGISS YLA	WYQQKPGKAPELLIY	AASTLQS	GVPSRFSGSGSGTDFTLTISCLQSEDFATYYC			QQYYSFP
	7	R6	**O4/O14**[29]/LFVK19H[*]/DPK11	DIQLTQSPSSLSASVGDRVTITC	RVSQGISS YLN	WYRQKPGKVPKLLIY	SASNLQS	GVPSRFSGSGSGTDFTLTISSLQPEDVATYYG			QRTYNAP
	7		**L22**[10]	DIQMTQSPSFLSASVGDRVSIIC	WASEGISS NLA	WYLQKPGKSPKLFLY	DAKDLHP	GVSSRFSGRGSGTDFTLTIISLKPEDFAAYYC			KQDFSYP
	7		**L23**[10]	AIRMTQSPFSLSASVGDRVTITC	WASQGISS YLA	WYQQKPAKAPKLFIY	YASSLQS	GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC			QQYYSTP
$V_\kappa II$	12	R8	**A2**[23]/DPK12	DIVMTQTPLSLSVTPGQPASISC	KSSQSLLHS DGKTYLY	WYLQKPGQPPQLLIY	EVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC			MQSIQLP
	13		**O1/O11**^{1}[29]/DPK13	DIVMTQTPLSLPVTPGEPASISC	RSSQSLLDSDDGNTYLD	WYLQKPGQSPQLLIY	TLSYRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC			MQRIEFP
	13		**O11**^{2}/V3a[29]	ASISC	RSSQSLLDSDDGNTYLD	CYLQKPGQSPQLLIY	TLSYRAS	GVPDRFSDSGSGTDFTLKISRVEAEDVGVYYC			MQRIEFP
	12		**L13**[10]	DIVMTQHLLSLPIPLGEPASISC	RSSQSLLHS DGNTYLD	WYLQKPGQSPQLLIY	TISNKFY	GVPNKFSGSRSGTGFTLKFSKVEAEDVGVYCC			EQGLQGP
	12		DPK14	EIVMTQTPLSLSTTPGEQASMSC	RSSQSLLHS DGYTYLY	WFLQKARPVSTLLIC	EVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDFGVYYC			MQDAQDP
	12	R0	**A3**[24]/**A19**[17]/DPK15	DIVMTQSPLSLPVTPGEPASISC	RSSQSLLHS NGYNYLD	WYLQKPGQSPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC			MQALQTP
	12		**A29**[17]/DPK27	DIVMTQTPPSLPVNPGEPASISC	RSSQSLLHS NGYTYLH	WYLQKPGQSPQLLIY	RVSNHLS	GVPDRFSGSGSGSDFTLKISWVEAEDVGVYYC			MQATQFP
	12		**A13**[17]	DIVMTQTPPSLPVNPGEPASISC	RSSQSLLHS NGYTYLH	WYPQKPGQSPQLLIY	RVSSRFS	GVPDRFSGSGSGSDFTLKISWVEAEDVGVYYC			MQATQFP
	12		**A23**[24]/DPK16	DIVMTQTPLSSPVTLGQPASISC	RSSQSLVHS DGNTYLS	WLQQRPGQPPRLLIY	KISNRFS	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC			MQATQFP
	12		**A7**[17]/DPK17	DIVMTQTPLSSPVTLGQPASISF	RSSQSLVHS DGNTYLS	WLQQRPGQPPRLLIY	KVSNRFS	GVPDRFSGSGAGTDFTLKISRVEAEDVGVYYC			TQATQFP
	12	R0	**A17**[17]/DPK18	DVVMTQSPLSLPVTLGQPASISC	RSSQSLVYS DGNTYLN	WFQQRPGQSPRRLIY	KVSNRDS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC			MQGTHWP
	12	R0	**A1**[17]/DPK19	DVVMTQSPLSLPVTLGQPASISC	RSSQSLVYS DGNTYLN	WFQQRPGQSPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC			MQGTHWP
$V_\kappa III$	8	R2	**A11**[24]/humkv305[27]/DPK20	EIVLTQSPATLSLSPGERATLSC	GASQSVSSS YLA	WYQQKPGLAPRLLIY	DASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC			QQYGSSP
	7		**L20**/Vg''[20]	EIVLTQSPATLSLSPGERATLSC	RASQGVSS YLA	WYQQKPGQAPRLLIY	DASNRAT	GIPARFSGSGPGTDFTLTISSLEPEDFAVYYC			QQRSNWH
	7	R3	**L16**[10]/humkv328/humkv328h2[34]	EIVMTQSPATLSVSPGERATLSC	RASQSVSS NLA	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC			QQYNNWP
	7	R0	**L2**[10]/humkv328h5[34]/DPK21	EIVMTQSPATLSVSPGERATLSC	RASQSVSS NLA	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC			QQYNNWP
	8	R0	**A27**[24]/humkv325/Vk RF[28]/DPK22	EIVLTQSPGTLSLSPGERATLSC	RASQSVSSS YLA	WYQQKPGQAPRLLIY	GASSRAT	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC			QQYGSSP
	8		**L25**[10]/DPK23	EIVMTQSPATLSLSPGERATLSC	RASQSVSSS YLS	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC			QQDYNLP
	8		**L10**^{1}[10]	EIVMTQSPPTLSLSPGERVTLSC	RASQSVSSS YLS	WYQQKPGQAPRLLIY	GASTRAT	GIPARFSGSGSGTDFTLTISSLQPEDFAVYYC			QQDYNLP
	8		**L10**^{2}/Vh[32]	EIVMTQSPPTLSLSPGERVTLSC	RASQSVSSS YLT	WYQQKPGQAPRLLIY	GASTRAT	SIPARFSGSGSGTDFTLTISSLQPEDFAVYYC			QQDHNLP
	7	R0	**L6**/Vg[32]	EIVLTQSPATLSLSPGERATLSC	RASQSVSS YLA	WYQQKPGQAPRLLIY	DASNRAT	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC			QQRSNWP
$V_\kappa IV$	13	R1	**B3**/VkIV[16]/DPK24	DIVMTQSPDSLAVSLGERATINC	KSSQSVLYSSNNKNYLA	WYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC			QQYYSTP
							?				
$V_\kappa V$	7	R0	**B2**/EV15[30]	ETTLTQSPAFMSATPGDKVNISC	KASQDIDD DMN	WYQQKPGEAAIFIIQ	EATTLVP	GIPPRFSGSGYGTDFTLTINNIESEDAAYYFC			LQHDNFP
$V_\kappa VI$	7		**A14**[26]/DPK25	DVVMTQSPAFLSVTPGEKVTITC	QASEGIGN YLY	WYQQKPDQAPKLLIK	YASQSIS	GVPSRFSGSGSGTDFTFTISSLEAEDAATYYC			QQGNKHP
	7	R1	**A10**[26]/**A26**[17]/DPK26	EIVLTQSPDFQSVTPKEKVTITC	RASQSIGS SLH	WYQQKPDQSPKLLIK	YASQSFS	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC			HQSSSLP
$V_\kappa VII$	11		**B1**[36]	DIVLTQSPASLAVSPGQRATITC	RASESVSFL GINLIH	WYQQKPGQPPKLLIY	QASNKDT	GVPARFSGSGSGTDFTLTINPVEANDTANYYC			LQSKNFP

Figure 3. Amino acid sequences of germ-line V_κ segments with open reading frames. Where possible, sequences have been assigned to their respective loci (bold type). Where two alleles have different nucleotide sequences these are followed by the superscripts ^{1} or ^{2}. Amino acid sequences from this study are labeled DPK1-DPK27 (see text). References for other sequences are in brackets, [*] indicates L. Foroni, personal communication. Amino acids are shown in single-letter codes. Sequences are arranged alphabetically within each subgroup according to the sequence of CDR1. Where two CDR1 sequences are identical, the order is based similarly upon the sequence of CDR2 or CDR3. The hypervariable regions, CDR1, CDR2 and CDR3 as defined by Kabat et al. [15] are labeled, as are the antigen-binding loops, L1, L2 and L3 as defined by Chothia and Lesk [50]. Numbering of amino acid residues is according to [15] except in CDR1 where numbering is according to [50]. The length of the L1 loop [50] is shown in italics. Germ-line sequences with known rearranged counterparts have been labeled R*N*, where *N* represents the number of amino acid differences between the germ-line V_κ sequence and its closest rearranged counterpart (see Table 2). Note that L5/L19^1/Vb/Vb'/V4b/DPK5 and L19^2/Vb''/DPK6; and L16/humkv328/humkv328h2 and L2/humkv328h5/DPK21 have identical protein sequences but different nucleotide sequences (see Fig. 4).

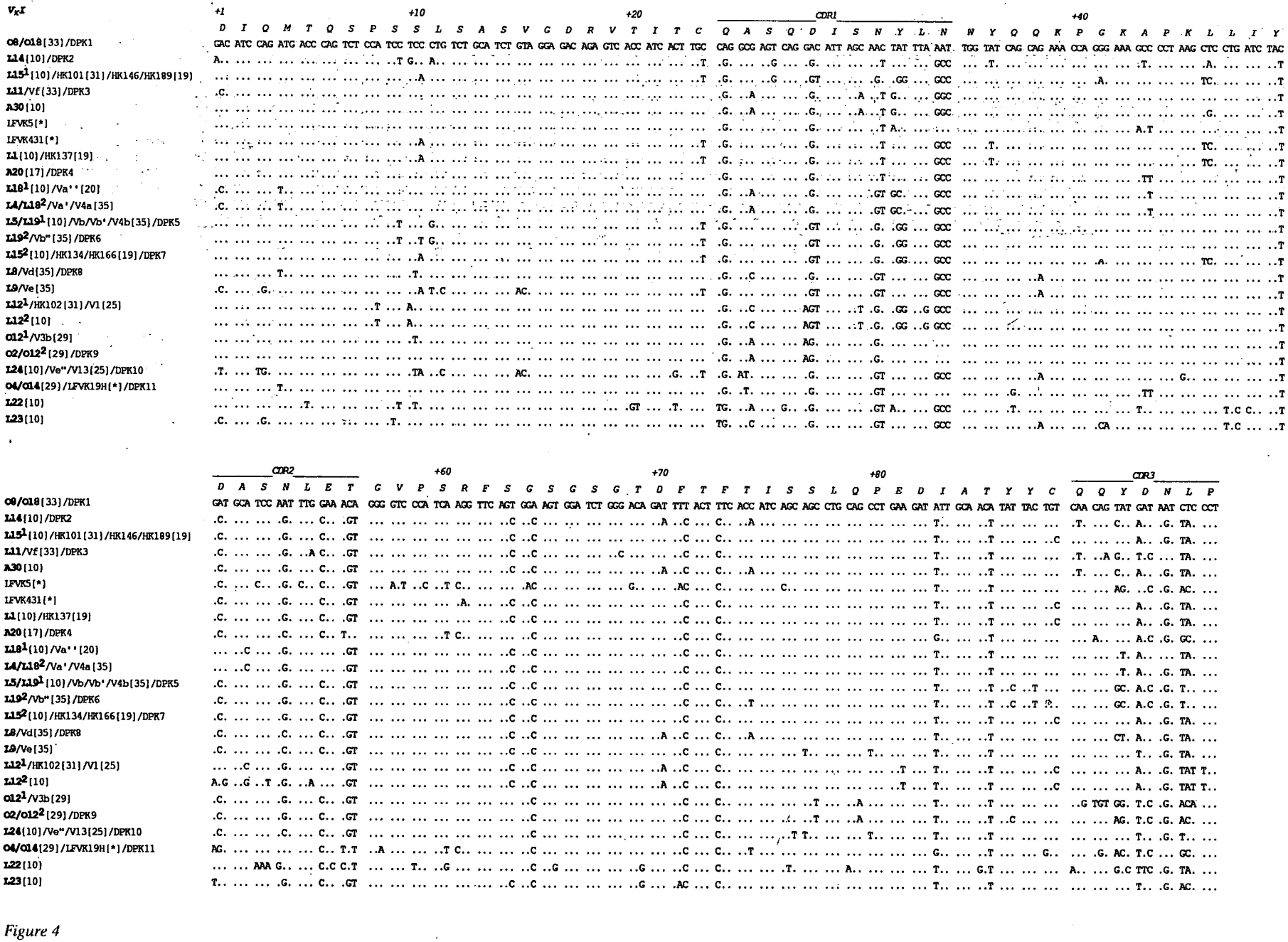
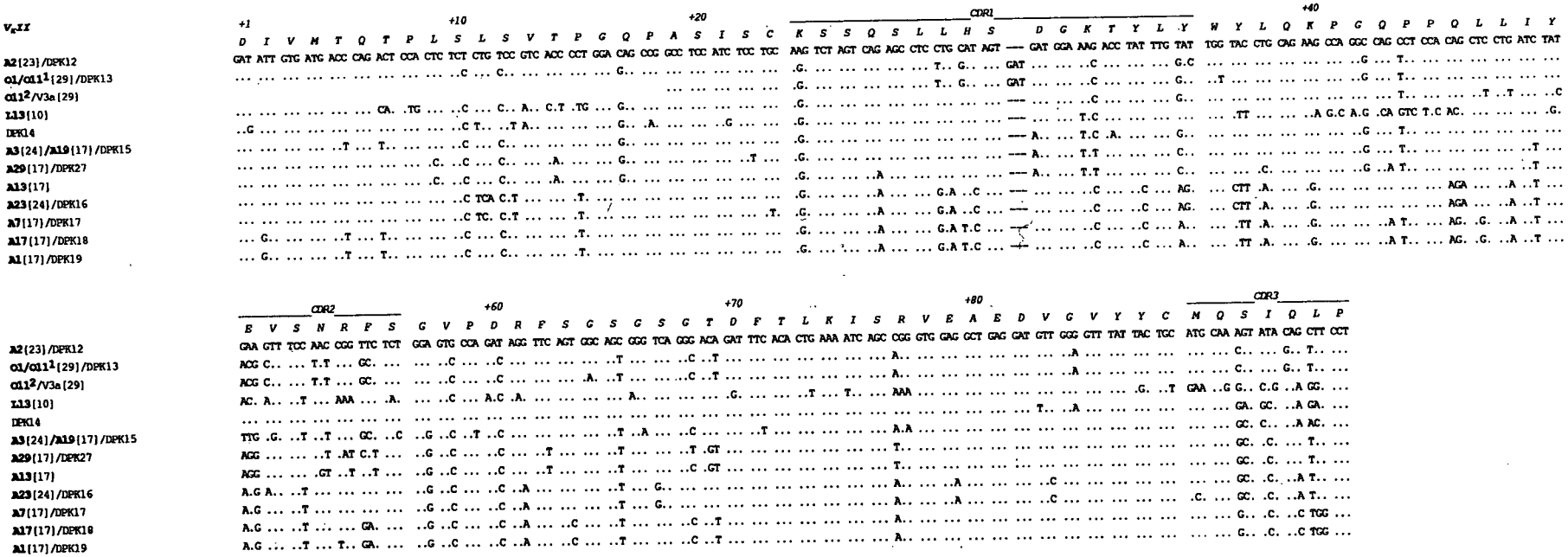
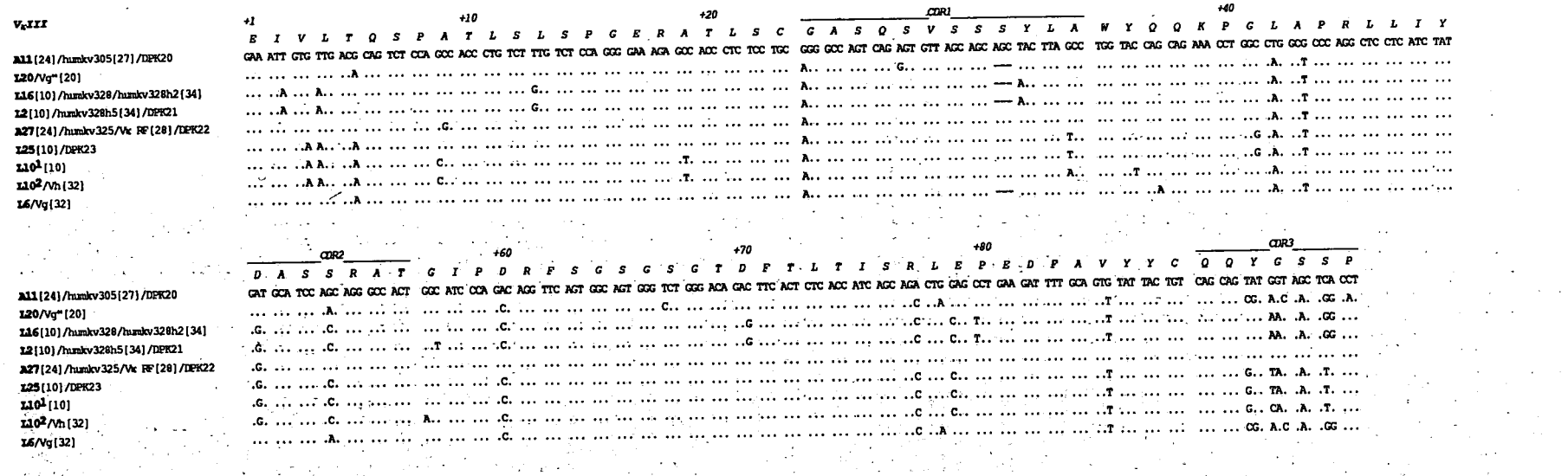

$V_\kappa I$
+1
+10
+20
CDR1
+40
D I Q M T Q S P S S L S A S V G D R V T I T C Q A S Q D I S N Y L N W Y Q Q K P G K A P K L L I Y
O8/O18[33]/DPK1
L14[10]/DPK2
L15[1][10]/HK101[31]/HK146/HK189[19]
L11/Vf[33]/DPK3
A30[10]
LFVK5[*]
LFVK431[*]
L1[10]/HK137[19]
A20[17]/DPK4
L18[1][10]/Va''[20]
L4/L18[2]/Va'/V4a[35]
L5/L19[1][10]/Vb/Vb'/V4b[35]/DPK5
L19[2]/Vb"[35]/DPK6
L15[2][10]/HK134/HK166[19]/DPK7
L8/Vd[35]/DPK8
L9/Ve[35]
L12[1]/HK102[31]/V1[25]
L12[2][10]
O12[1]/V3b[29]
O2/O12[2][29]/DPK9
L24[10]/Ve"/V13[25]/DPK10
O4/O14[29]/LFVK19H[*]/DPK11
L22[10]
L23[10]
CDR2
+60
+70
+80
CDR3
D A S N L E T G V P S R F S G S G S G T D F T F T I S S L Q P E D I A T Y Y C Q Q Y D N L P
O8/O18[33]/DPK1
L14[10]/DPK2
L15[1][10]/HK101[31]/HK146/HK189[19]
L11/Vf[33]/DPK3
A30[10]
LFVK5[*]
LFVK431[*]
L1[10]/HK137[19]
A20[17]/DPK4
L18[1][10]/Va''[20]
L4/L18[2]/Va'/V4a[35]
L5/L19[1][10]/Vb/Vb'/V4b[35]/DPK5
L19[2]/Vb"[35]/DPK6
L15[2][10]/HK134/HK166[19]/DPK7
L8/Vd[35]/DPK8
L9/Ve[35]
L12[1]/HK102[31]/V1[25]
L12[2][10]
O12[1]/V3b[29]
O2/O12[2][29]/DPK9
L24[10]/Ve"/V13[25]/DPK10
O4/O14[29]/LFVK19H[*]/DPK11
L22[10]
L23[10]

Figure 4





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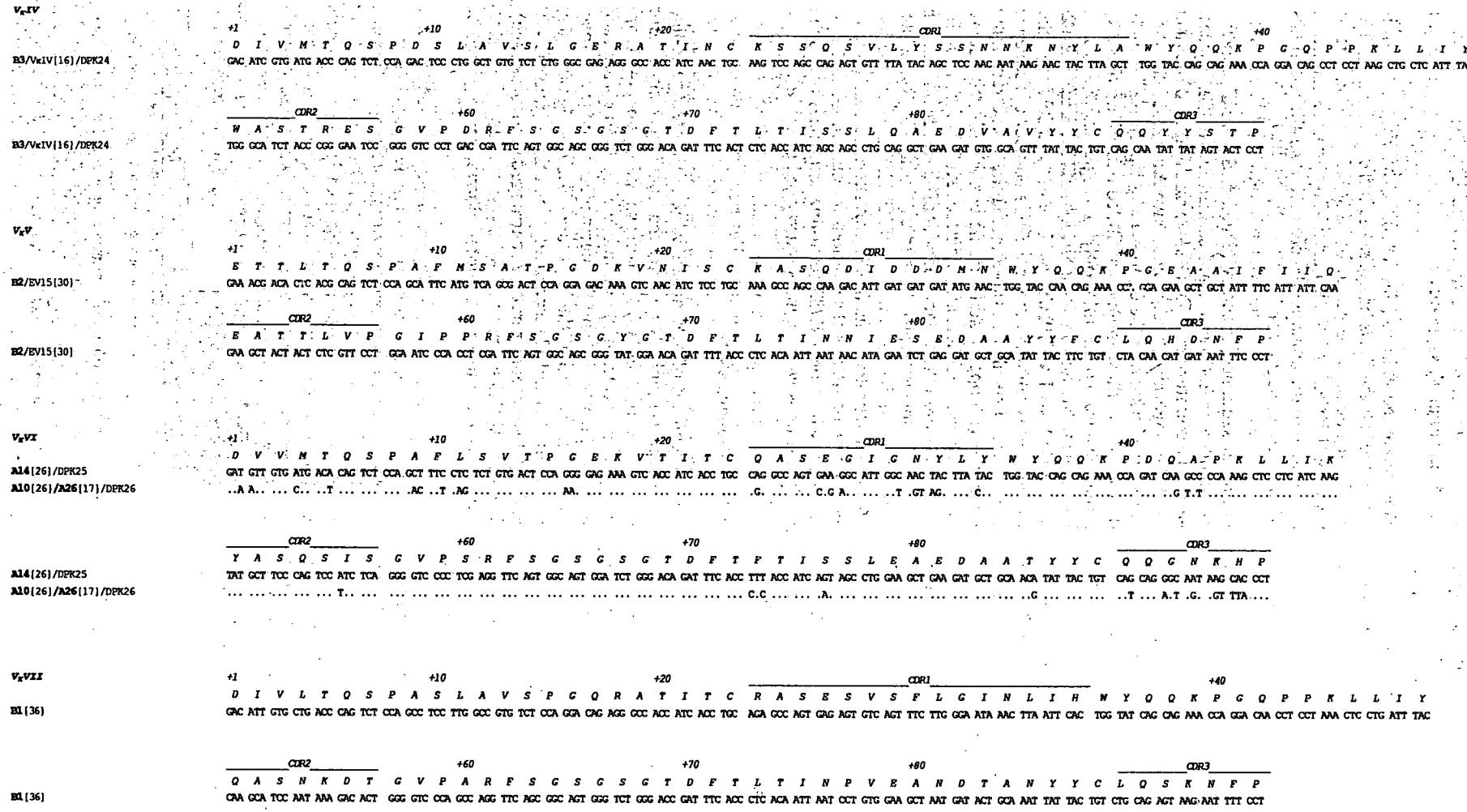

Figure 4. Nucleotide sequences of germ-line V_κ segments with open reading frames. Sequences were extracted from the Genbank/EMBL nucleotide databases wherever possible. Sequences from this study have been labeled with their DPK codes (see text). References for other sequences are in brackets. [*] denotes L. Foroni, personal communication. A translation of the master sequence is shown directly above it: residues are numbered according to [15]. Nucleotides identical to the master sequence are represented by a dot and insertions are represented by a dash. The directory of human germ-line V_κ sequences is available on disk on request.

A4 [25], A18 [19], A22 [24], L4 (Va) [35], HK100 [31] and to the orphon segments W2 [38], W8/W10 [38], Z2 [43], chr22-4 [41]: DPK37 is new and is most similar to the orphon Z3 [41] (deletion of one nucleotide and three changes). The sequences of the four new segments, DPK2, DPK14, DPK23 and DPK37, were confirmed with clones from independent PCR amplifications. Subsequently, two of these segments, DPK2 and DPK23, proved to be identical to the sequences L14 and L25, which were published after the submission of this paper [10]. The fact that 35 of the 37 segments amplified from DP are identical to published sequences confirms that germ-line V_κ sequence polymorphism is rather limited [46].

In addition to the confirmed sequences, many clones had sequences which were not observed in independent amplifications. Some had one or two nucleotide differences from the V_κ segments DPK1-DPK37 and were probably due to PCR errors. The 21 nucleotide substitutions in the 52 V_κI sequences correspond to 5×10^{-5} changes per nucleotide per PCR cycle and are consistent with the error rate for Taq polymerase [47]. Other clones appeared to be due to PCR cross-over [8, 48].

We have compiled a comprehensive directory of the amino acid and nucleotide sequences of all germ-line V_κ segments with open reading frames (Figs. 1, 3 and 4). Where possible, sequences have been assigned to their respective loci. We have compared these sequences with those of 113 rearranged V_κ sequences taken from the Genbank/EMBL nucleotide databases and 123 additional sequences from the literature (our rearranged database is available on request). All rearranged sequences were assigned to their closest germ-line counterparts. In Table 2 we note the closest sequence identities and the number of rearranged sequences assigned to each germ-line sequence. In a few examples, the rearranged sequence was found to be a composite of two V_κ segments, presumably arising by PCR cross-over during cloning. For example, V_κI-X14 [49] is identical to O4/O14/LFVK19H/DPK11 over the first 220 nucleotides and virtually identical to O2/O12[2]/DPK9 over the last 100 nucleotides, due to a cross-over in FR3.

Only 24 of the 50 germ-line sequences in our directory were found to have rearranged counterparts. Although 162 rearranged sequences correspond to mapped V_κ segments from the J_κ-proximal region of the kappa locus only 5 are derived from the J_κ-distal region. These correspond to the germ-line sequences A2/DPK12, A1/DPK19, A11/humkv-305/DPK20 and L16/humkv328/humkv328h2 (see Fig. 1). Of the rearranged sequences, 69 correspond to O8/O18/-DPK1, L5/L19/Vb/Vb'/V4b/DPK5, O2/O12[2]/DPK9, O4/-O14/LFVK19H, A3/A19/DPK15 and A10/A26/DPK26: in these cases each rearranged sequence could have been derived from V_κ segments from either the J_κ-proximal or J_κ-distal region. No rearranged sequences correspond to the unmapped V_κ segments LFVK5, LFVK431 and DPK14.

The distribution of the number of amino acid differences across all 236 assigned rearranged sequences is shown in Fig. 5. This includes sequences of antibodies with a wide range of specificities. As sequence polymorphism at the germ-line level is limited (see above) the majority of changes are probably due to somatic mutation.

4 Discussion

4.1 Functional V_κ segments

The map of the V_κ locus (Fig. 1) contains 76 segments. It is estimated, on the basis of hybridization experiments, that 4–6 V_κ segments have yet to be mapped [11, 14]. The V_κ segments LFVK5, LFVK431 and DPK14 could, therefore, correspond to the unmapped loci or could be allelic variants of mapped V_κ segments.

From our sequence directory (Fig. 3 and 4) and from the assignment of 236 rearranged sequences to their closest germ-line counterparts (Table 2) we find that no more than 24 of the 50 germ-line sequences have rearranged counterparts, suggesting that the expressed V_κ repertoire is mainly derived from these segments. Indeed for 7 of these V_κ sequences we could find only a single example of a rearrangement, and in 4 cases (L1/HK137, L12[1]/HK102/V1, O4/O14/LFVK19H/DPK11 and A2/DPK11) the assignment is tentative in view of the number of sequence differences. It is also possible that some of the remaining segments with open reading frames are functional, but are only rarely used. Others may well prove to have frame shifts or stop codons in the leader exon, defective recombination signals or splice sites or other *cis*-acting defects which render them non functional. A14/DPK25, for example, has an altered heptamer which may prevent its rearrangement. We have made similar observations for human germ-line V_H segments where only 49/83 germ-line sequences with open reading frames were seen as rearranged genes [8].

4.2 V_κ segment usage

The assignment of rearranged V_κ genes to their germ-line counterparts (Table 2) confirms that segments from all four clusters (A, B, L and O) and from six subgroups (I–VI) are used. Segments from the V_κI and V_κIII subgroups are used most frequently (96 and 90 rearranged sequences respectively); those from the V_κII and V_κIV subgroups less frequently (24 and 22 respectively); and those from the V_κV and V_κVI subgroups rarely (2 rearranged sequences each). The only V_κVII segment, B1, has no rearranged counterpart in our database. As noted above, some of the V_κ

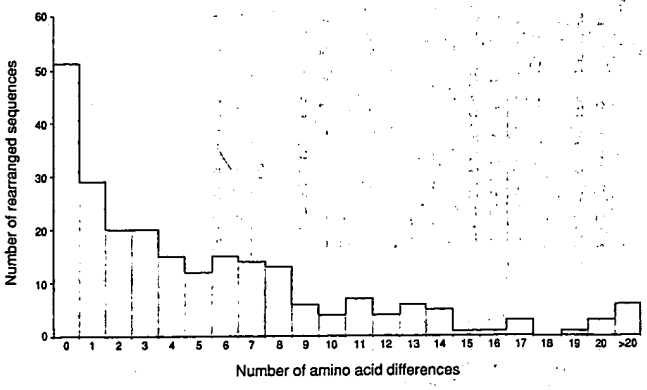

Figure 5. Distribution of the number of amino acid differences between each sequence in a database of rearranged V_κ sequences (236 in total) and its closest germ-line counterpart in our directory. The database of rearranged V_κ sequences is available on disk on request.

segments are rarely used, and of the 24 germ-line sequences, only about 11 appear to be frequently used (Table 2).

Of the rearranged sequences, 162 correspond to V_κ segments from the J_κ-proximal region of the kappa locus, whereas only 5 appear to be derived from the J_κ-distal region. Indeed, one of these rearranged sequences from the J_κ-distal region, EVJK11, which corresponds to A11/humkv305/DPK20, is the product of aberrant recombination [30]. An additional 69 rearranged sequences could conceivably be derived from V_κ segments in either the proximal or distal regions. However, as has been previously noted, the segments O2 (distal) and O12 (proximal) differ by one nucleotide in a region which flanks the exon [29]: rearranged sequences which include this region appear to be derived from O12 rather than O2 [29]. Although there is a clear bias towards use of the J_κ-proximal V_κ segments, we can detect no bias within the J_κ-proximal region.

The reason for the remarkable bias towards use of V_κ segments from the J_κ-proximal region is unclear. It may be due to the relative distances of the proximal and distal regions from the J_κ segments or the different recombination mechanisms necessary to produce the rearranged V_κ gene: most V_κ segments in the proximal region rearrange by deletion, whereas those in the distal region must rearrange by inversion. We note that lack of the J_κ-distal copy of the locus (haplotype 11) does not appear to be deleterious [21].

The assignment of rearranged V_κ genes may help in dissecting the mechanisms of the human immune system. For example, more than half of the rearranged sequences in our database have three or fewer residue changes (Fig. 5). Antibodies from patients with chronic lymphocytic leukemia (CLL) and X-linked agammaglobulinemia (XLA) are rarely mutated or unmutated. More highly mutated V_κ genes tend to be seen in myelomas and in antibodies which are subject to an antigen-driven response. In the case of autoantibodies, the level of somatic mutation appears to be normal.

4.3 Structures of loops implicit in the human V_κ segments

The antigen-binding loops of immunoglobulin variable domains have been shown to adopt a limited number of main chain conformations or "canonical structures" [50, 51]. The structure of each loop depends on its length and the identity of certain key residues involved in its packing, and using this information it is sometimes possible to predict the structure of the loops from the sequence of the V_κ domain [51]. Here we have attempted to identify the loop structures of those human V_κ segments that appear to undergo rearrangement (Table 2).

Across different species, the V_κ antigen binding site loop L1 (residues 26–32, corresponding to CDR1, see Fig. 3) can have lengths of 6, 7, 8, 11, 12 and 13 residues and presumably can form at least six major conformations [50, 51]. The structures of antibodies with loops of 6, 7, 12 and 13 residues have been solved crystallographically: in each case residue 29 in the loop is buried in the β-sheet framework, and packs against residues 2, 25, 33 and 71 [50, 51]. These packing contacts are often very similar: Ile/Val 2, Ala/Ser 25, Val/Ile/Leu 29, Leu/Met 33 and Tyr/Phe 71 [50].

However, for those human V_κ segments with rearranged counterparts (Table 2), we only see L1 loops of 7, 8, 12 and 13 residues (Fig. 3). In all cases the packing contacts are highly conserved, suggesting that the segments should encode four major conformations of the L1 loop. Of the germ-line sequences, 17 (141 rearranged sequences) encode a 7-residue L1 loop, 2 sequences (49 rearranged sequences of which 48 use the V_κIII segment A27) encode an 8-residue loop, 4 sequences (24 rearranged sequences) encode a 12-residue loop and 1 sequence (22 rearranged sequences) encodes a 13-residue L1 loop. L1 lengths of 6 residues have only been seen in mice [15], suggesting that mouse V_κ segments encode structures which cannot be encoded by human V_κ segments.

The L2 loop (residues 50–52, corresponding to CDR2, see Fig. 3) is only three residues long in all structures, and undergoes packing interactions with framework residues 48 and 64 [50, 51]. All germ-line V_κ sequences with rearranged counterparts (Fig. 3 and Table 2) have Ile 48 and Gly 64, indicating that they are likely to encode a single loop conformation.

The L3 loop (residues 91–96, corresponding to CDR3, see Fig. 3) is encoded mainly by the V_κ segment, and is most often a six-residue loop with Gln, Asn or His at 90 and Pro at 95 [50, 51]. Almost all human germ-line V_κ sequences with rearranged counterparts encode Gln 90 and Pro 95, except A20/DPK4 (Lys 90), O4/O14/LFVK19H/DPK11 (Arg 90), $L12^1$/HK102/V1 and $L12^2$ (Ser 95). This indicates that most human V_κ germ-line segments encode a single conformation of this loop: but depending on the location of the V-J join and nucleotide addition, other conformations may be formed in the rearranged gene.

We note that several of the germ-line sequences that do not have rearranged counterparts in our database (Fig. 3 and Table 2) have atypical residues at the key residues involved in the packing of the antigen-binding loops (L1 loop: L22 [Leu 48]; LFVK5 and $O11^2$/V3a [Asp 64]; L3 loop: L20/Vg" [His 95]) or unusual loop lengths (B1 has an L1 of 11 residues).

In combination, the three antigen-binding loops L1, L2 and L3 of human germ-line V_κ segments are likely to encode four major folds. Since our repertoire of cloned human germ-line V_κ segments includes 15 of the 24 germ-line V_κ sequences with rearranged counterparts and examples of each of the four major folds it should be a valuable resource for building synthetic antibodies for use in phage display libraries.

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